

Can we improve the prediction of complications and outcome in aneurysmal subarachnoid hemorrhage? The clinical implications of serum proteomics

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Aneurysmal subarachnoid hemorrhage is a devastating condition, often leading to a debilitating outcome. Delayed ischemic neurological deficits are considered the feared sequelae. Proteomics is a large-scale study of proteins incorporating structural and functional properties in complex biological fluids. Analysis of proteomes has led to identifying relevant complex proteins related to specific pathophysiological processes reflecting the severity and extent of diseases. Proteomics has evolved in the past few years; more biomarkers are deemed clinically relevant to diagnose, monitor, and define prognosis in patients with aneurysmal subarachnoid hemorrhage. Despite the absence of candidate biomarkers in the clinical routine, many have shown promising results. The complexity of proteins implicated in aneurysmal subarachnoid hemorrhage rendered these biomarkers' clinical use paved with various pitfalls and technical difficulties, especially when data about the perfect timing and values are lacking. We review the latest literature concerning serum proteomics and their clinical utility regarding the prediction of cerebral vasospasm and other complications of aneurysmal subarachnoid hemorrhage, as well as the clinical outcome. Future prospective studies will allow changing the disease's course, label patients according to their prognosis to provide earlier and better management and improve outcomes.

Keywords

Proteome; aneurysm; subarachnoid hemorrhage; vasospasm; delayed cerebral ischemia

1. Introduction

Among the vast etiologies of strokes, aneurysmal subarachnoid hemorrhage (aSAH) accounts for about 2-5% of all stroke cases (Lovejoy et al., 2010). The occurrence of multiple post-hemorrhagic complications such as the initial parenchymal damage of the subarachnoid hemorrhage (De Marchis et al., 2015; Eriksson et al., 2019; Hartings et al., 2017; Sarrafzadeh et al., 2010), delayed cerebral ischemia (DCI), hydrocephalus, epilepsy, and

systemic infections, constitute the reason for poor outcome after aSAH (Frontera et al., 2008). DCI is the main predictor of clinical outcome in aSAH and was believed to come secondary to cerebral vasospasm (CVS) in 20-30% of cases (Haley et al., 1992). More recently, it is found to be a more complex multifactorial phenomenon. A spreading depolarization precedes the decreased cerebral blood flow and partial tissue pressure of oxygen in the microcirculation, which leads to stroke and subsequent cytotoxic edema (Lückl et al., 2018). This acute disturbance in the microcirculation can be merely due to neuronal and glial depolarization, which release high potent vasoconstrictors (Dreier, 2011). This was deduced from the alternating patterns of hyperperfusion-hypoperfusion during the acute depolarization waves (Lückl et al., 2018; Minhas et al., 2003), which cannot be the mere result of sustained vasoconstriction of large arteries (Minhas et al., 2003). The most used scales to predict prognosis and outcome in patients with aSAH are Hunt and Hess (1968) and the World Federation of Neurological Surgeons (WFNS). Since both are clinical scores and given the dire consequences of aSAH complications, there is a need for an objective test that is non-invasive, simple and reliable, predicting these complications and improving the timely delivery of appropriate treatment.

Serum biology involves genomics/transcriptomics, proteomics, glycomics, lipidomics, metabolomics and degradomics (Jean Beltran et al., 2017). Most recent data has demonstrated the presence of a metabolic crisis correlating with various clinical outcomes, and temporal correlations have been confirmed as early as 15 minutes after subarachnoid hemorrhage (Gewirtz et al., 1999).

The term proteomics describes the study of a complete set of proteins existent in a cell, organ, or organism at a specific time (Wilkins et al., 1996). It's a large-scale analysis of proteins, found as multiprotein complexes, or all proteins encoded by the genome, constituting the proteome (Pandey and Mann, 2000). Biomarker discovery is a very challenging procedure due to the complexity of the body fluids and the wide variety of protein concentrations found (Seibert, 2005). Significant workup has been done regarding the identification, validation, and clinical correlation of different protein biomarkers to measure the severity of strokes and trau-

matic brain injury (Mrozek et al., 2014). Protein micro identification and sequencing have evolved substantially using mass spectrometry, a very sensitive analytical method (Shevchenko et al., 1999). Fig. 1 presents the location of serum proteomics involved in aSAH. It also highlights the consequences of disrupting the blood-brain barrier and its implications (Lublinsky et al., 2019). It is important to find proteomes that can be deemed predictors of DCI and other complications of aSAH to treat them timely and correctly. None of these biomarkers have reached routine clinical use due to a lack of enough integrated data about specific timing, techniques, and clinical significance. This article reviews recent studies regarding serum proteomics as mediators of the disease and predictors of complications and outcome, as a bridge towards diagnostic workup and treatment guidance. Results are summarized in Table 1 and Table 2.

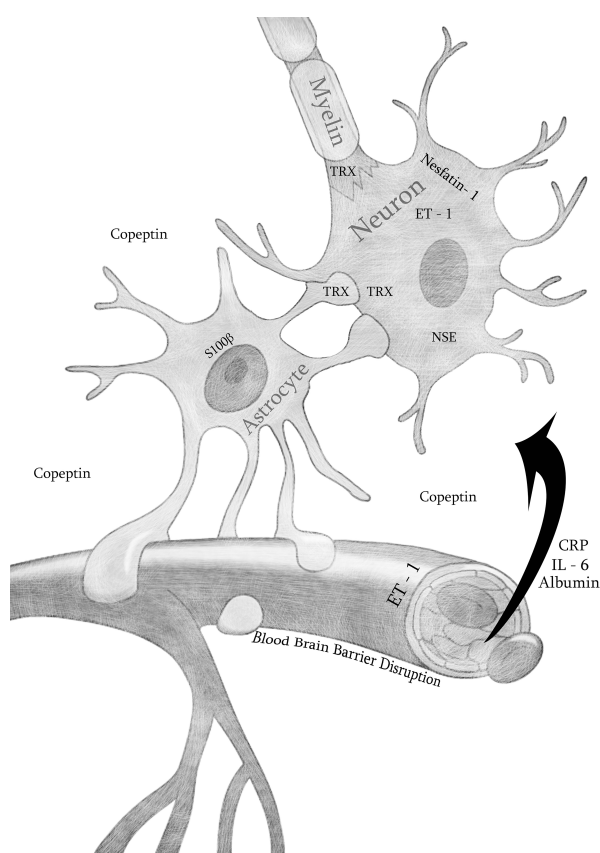


Fig. 1. A schematic illustration showing proteomes related to aSAH, and their location inside neurons, astrocytes, and blood vessel. The disruption of the blood-brain barrier allows the pouring of biomarkers with subsequent inflammation of the surrounding brain parenchyma. ET-1: Endothelin-1. TRX: Thioredoxin. NSE: Neuron-specific enolase. IL-6: Interleukin-6. CRP: C-reactive Protein.

2. Endothelin-1 (ET-1)

The most potent endogenous vasoconstrictor, endothelin-1, is a 21 amino acid peptide produced by endothelial cells (Suhardja, 2004). Most of the studies regarding endothelin-1 levels and its prediction of cerebral vasospasm were done on Cerebrospinal

Fluid (CSF) samples. Very few articles mentioned plasma ET-1 as a potential predictor of vasospasm and DCI, but most of them were descriptive or done on animals. A study involved 70 patients with a median age of 45.8 years, ET values were measured in samples obtained within 24 hours either before or after angiography were higher in patients with angiographic evidence of severe vasospasm (2.7 ± 0.4 pg/mL in 13 patients compared with 2 ± 0.2 pg/mL in 34 patients; $P = 0.078$); the values were also higher in patients with angiographic evidence of diffuse moderate-to-severe vasospasm (2.6 ± 0.3 pg/mL) than in others (Juvela, 2000). Since plasma ET-1 shows promising results, more studies should be done on plasma levels since it will be more feasible, especially in critically ill patients.

Endothelin receptor antagonist (ETRA) showed the promising result to prevent CVS but failed to show a benefit in term of mortality and neurological outcomes (Kramer and Fletcher, 2009; Ma et al., 2012; Macdonald et al., 2011; Vergouwen et al., 2012), which suggests that additional factors contribute to clinical deterioration and DCI, the main predictor of outcome. This strongly challenges the concept that angiographic vasospasm is the predominant factor for DCI. In particular, endothelin receptor antagonist was given in 5 trials with 2601 patients (Vergouwen et al., 2012). It showed an increased poor functional outcome despite CVS reduction; no effect was noticed regarding DCI, cerebral infarction, or case fatality rate. This emphasizes the various etiologies of DCI rather than to be narrowed down to CVS.

3. Interleukin-6

Interleukin-6 is a pleiotropic cytokine that plays a role in vascular and metabolic diseases (Hunter and Jones, 2015). IL6R(CD126), gp130(CD130), GTPase Ras-Raf, mitogen-activated protein kinases (MAPK), and Janus kinases (JAKs) signal transducer and activator of transcription constitute the signaling pathway of this cytokine with hormone-like activity (Hunter and Jones, 2015). IL-6 pro-inflammatory and anti-inflammatory property depending on the pathogenesis involved (Hunter and Jones, 2015). In aSAH, il-6 has a neuroinflammatory response (Suzuki et al., 2009).

The proteolytic processing of the IL-6 receptor on neutrophils turns on the signaling pathway in residing cells such as cerebral vasculature cells (Jones, 2005). IL-6 levels were positively linked to poor outcomes according to several studies (Höllig, Rummel et al., 2015; Höllig, Thiel et al., 2015; Kao et al., 2015; Muroi et al., 2013). We mention a detailed study that involved 80 aSAH patients. IL-6 serum levels were significantly raised in patients presenting with low Hunt & Hess (H&H) grade and patients with poor clinical outcome (Glasgow outcome scale 1-3), reflecting the inflammatory component of severe aSAH (Chaudhry et al., 2017). In a multivariate setting, serum IL-6 was independently associated with poor outcome assessed by mRS (modified Rankin Scale). More elevation in IL-6 serum level was noted in patients with concomitant intraventricular/intracranial bleed, hydrocephalus, and seizure (Chaudhry et al., 2017).

The role of inflammatory pathways in developing cerebral vasospasm and Delayed Ischemic Neurologic Deficit (DIND) was reflected with higher IL-6 level in the serum of these patients on day 3, 7 and 9, with levels approaching 1.5 ± 0.2 (log IL-6 pg/mL),

Table 1. Proteomics that are significantly related to clinical vasospasm and DIND, with their serum value and time of sample collection.

<i>Biomarker</i>	<i>study/year</i>	<i>low risk of vasospasm and DIND</i>	<i>high risk of vasospasm and DIND</i>	<i>time of sample taken</i>
<i>Endothelin-1</i>	Juvela (2000)	median 2 pg/mL	median 2.7 pg/mL	within 24 hours from admission
<i>Nesfatin-1</i>	Wei et al. (2018)	Median 8.84 ng/mL	Median 11.73 ng/mL	On admission
<i>C-reactive protein</i>	Romero et al. (2014)	Mean 3 mg/L	Mean 16 mg/L	Between 3 rd and 4 th day
<i>Myeloperoxidase</i>	Lim et al. (2012)	Mean 59.4 ng/mL	Mean 115.5 ng/mL	2 or more days prior to vasospasm
<i>Interleukin-6</i>	Chaudhry et al. (2017)	Mean 1.0 ± 0.2 (log IL-6 pg/mL)	Mean 1.5 ± 0.2 (log IL-6 pg/mL)	Days 3 7 9 post aSAH

Table 2. Proteomics is significantly related to a bad outcome, with their serum value and time of sample collection.

<i>Biomarker</i>	<i>study/year</i>	<i>Good outcome</i>	<i>bad outcome</i>	<i>time of sample taken</i>
<i>Nesfatin-1</i>	Wei et al. (2018)	Median 9.11 ng/mL	Median 11.34 ng/mL	On admission
<i>Thioredoxin</i>	Dai et al. (2016)	< 25.1 ng/mL	> 25.1 ng/mL	On admission
<i>C-reactive protein</i>	Romero et al. (2014)	Mean 4 mg/L	Mean 8-10 mg/L	Between 3 rd and 4 th day
<i>Copeptin</i>	Fung et al. (2013)	Median 6.8 pmol/L	Median 26.3 pmol/L	At admission
<i>Albumin</i>	Kapoor et al. (2018)	> 3.5 mg/dL	< 3.5 mg/dL	At admission
Neuron-specific enolase	Tawk et al. (2016)	< 15 ng/mL	> 15 ng/mL	Within a mean of 25.2 hours
<i>S100B</i>	Moritz et al. (2010)	Mean < 0.17 µg/L	Mean > 0.17 µg/L	Sampling during 15 days post aSAH
<i>Interleukin-6</i>	Chaudhry et al. (2017)	Mean 1.0 ± 0.2 (log IL-6 pg/mL)	Mean 1.5 ± 0.2 (log IL-6 pg/mL)	Days 3 7 9 post aSAH

compared with patients without DIND or vasospasm with serum IL-6 level 1.0 ± 0.2 (log IL-6 pg/mL) (Chaudhry et al., 2017).

Another study involved 24 patients with aSAH, where IL-6 serum levels were compared according to different intracranial pressure post-bleed. IL-6 levels were higher in patients with high intracranial pressure 5-9 days after SAH, indicating a severe inflammatory reaction. It was also correlated with low GOS levels, especially on 4-9 days post-aSAH (Graetz et al., 2010). Similar results were also found in another study, where CSF, but not plasma IL-6 levels, were positively correlated with DCI development, with concomitant C-reactive protein response. CSF levels were higher than plasma levels, which may be related to the smooth muscle production of IL-6, which reflects the vasospasm ensuing after aSAH (Sarrafzadeh et al., 2010).

Interventions by pharmacotherapy for IL-6 signaling interruptions reversed vasospasm in femoral artery vasospasm in a rat model (Bowman et al., 2004). This can bring us closer to achieve a better understanding of IL-6 role in the severity of aSAH and developing subsequent targeted therapy to reduce the complications.

In contrast to neuroinflammatory activation, immunodepression was introduced in various neurological conditions such as stroke and neurotrauma. (Prass et al., 2003; Woiciechowsky et al., 1998) Immunodepression was evident in aSAH. There were significantly lower counts of CD4 T cells, CD8 T cells, and natural killer cells as early as day 2 in symptomatic aSAH than asymptomatic aSAH. This has led to more infection rates -particularly

pneumonia- in patients with symptomatic aSAH (Sarrafzadeh et al., 2010). This is important to mention since proteomics biomarkers can be minimal in these cases, requiring the implementation of preventive measures to avoid infections post aSAH.

4. Nesfatin-1

Nesfatin-1 is an anorexigenic peptide, combining calcium and Nucleobindin2, localized in the CSF and paraventricular nucleus of the hypothalamus (Wei et al., 2018). A study included 97 patients with aSAH. Those with poor outcomes and higher H&H grades had higher levels of nesfatin-1 than patients with good outcomes (11.342 ± 1.826 vs. 9.118 ± 0.278 ng/mL). Also, patients with DCI had higher levels of nesfatin-1 than patients with non-DCI (11.736 ± 1.844 vs. 8.842 ± 2.629 ng/mL) (Luo et al., 2019). Another study found that when 20 g/kg nesfatin-1 given to rats with induced SAH, they had better outcomes concerning neurological impairment and oxidative brain injury (Tang et al., 2012). On the other hand, there was an emphasis on the neuroprotective property of nesfatin-1 when injected in rats with subarachnoid hemorrhage-induced brain damage (Özsavcı et al., 2011). This seemingly contradictory result can be explained by the vasoconstrictive effect of nesfatin-1 as a defense mechanism against aneurysms or a rupture stimulant, as well as having an anti-apoptotic and anti-inflammatory effect (Cakir et al., 2017).

5. Thioredoxin (TRX)

TRX is a 12.5 kDa ubiquitous intracellular thiol protein involved in redox reactions. It modulates inflammation via its antioxidant property, in addition to its implication in cell growth and apoptosis (Koháryová and Kollárová, 2015). TRX has recently been considered a surrogate marker for severity and poor outcome in intracerebral hemorrhage, head trauma and ischemic strokes (Pan et al., 2016; Qi et al., 2015; Qian et al., 2016). A study found a significant elevation of TRX in 132 patients with aSAH (23.4 ± 12.2 ng/mL), compared with 132 controls (8.5 ± 4.0 ng/mL) as association between serum TRX levels and modified Fischer score upon admission and 6 months' predictor of mortality and unfavorable outcome. The optimal cut-off value of serum Trx levels as a predictor for 6-month mortality was considered to be 25.1 ng/mL (Dai et al., 2016). TRX levels improved the predictive performance of GCS score for 6 months' outcome with a cutoff value of 20.3 ng/ml, but not for 1-week mortality or 6 months mortality when 112 patients with aSAH and 112 controls were studied (Pan et al., 2016).

TRX has reflected the oxidative stress that occurs during subarachnoid hemorrhage. Both studies mentioned above had well correlated with prediction of the outcome when TRX was taken upon admission. It constitutes an excellent predictive value in terms of outcome, yet no data have been mentioned regarding vasospasm and DCI prediction.

6. Creactive protein

CRP is an inflammatory marker of the pentraxin family, well known for its sensitivity. Its synthesis in hepatocytes highly depends on IL-6 and IL-1 (Mazlam and Hodgson, 1994). Many studies have demonstrated that an elevated CRP level can predict cerebral vasospasm and, subsequently, DIND (Fountas et al., 2009). Similarly, patients who presented with high Fischer and H&H grades on admission have developed higher CRP levels in serum (6-7 mg/L) (Romero et al., 2014).

The more unsatisfactory outcome expressed in GOS was correlated with higher serum CRP levels, which peaked on the 3rd and 4th days post aSAH (8-10 mg/L) (Romero et al., 2014). Patients with clinical vasospasm had CRP levels reaching 16 mg/L (Romero et al., 2014).

CRP findings in aSAH correspond to the possible prediction of the clinical outcome. It reflects the severity of aSAH by being a surrogate of inflammation. Yet, it is confounded that many of these patients had concomitant systemic infections or other conditions that could elevate CRP levels. CRP will not be a specific marker during aSAH, although its sensitivity is well proved.

7. Copeptin

A protein released as an equimolar ratio with vasopressin from the same precursor protein, preprovasopressin, consists of 164-amino acid (Morgenthaler et al., 2006). Since vasopressin is involved in the potentiation of corticotrophin-releasing hormone, which increases Adrenocorticotrophic hormone, it leads to cortisol production. Hence, Copeptin -the surrogate marker for vasopressin release- is considered a stress response marker (Morgenthaler et al., 2006). Many studies chose Copeptin due to its stability that remains intact for days, thus allowing easy blood sample measurement (Morgenthaler et al., 2006).

Median copeptin levels in healthy individuals have been reported and range from 3.7 to 4.2 pmol/L (Katan et al., 2009). A study found a significant association between higher copeptin level and WFNS grade 4 and 5 with a median of 26.3 pmol/L, Fischer grade 3 with 18.3 pmol/L, and ICH (intracerebral hemorrhage) with 52.5 pmol/L, along with an association with the amount of subarachnoid blood. Nevertheless, it has been concluded that admission copeptin levels are not reliable for predicting vasospasm or delayed cerebral ischemia (Fung et al., 2013).

Zhu et al. (2011) and colleagues reported a correlation between the elevated serum copeptin levels with vasospasm during the subacute period and increased mortality at 1 year, but should not be used since clinical grades were found to have higher accuracy.

8. Albumin

Albumin is a negative acute phase reactant, known to fall acutely due to metabolic response to injury. Low nutritional status is well known for critical illness and unfavorable prognosis (McCluskey et al., 1996). Considering albumin, a neuroprotective protein, Kapoor and colleagues found that patients having lower albumin (< 3.5 mg/dL) levels at admission had higher rates of neurological deficits, infarcts, mortality, unfavorable GOS, H&H grade and Fisher grade. Also, at 1 week, a fall in albumin level compared to the admission level was significantly linked to unfavorable GOS and mortality (Kapoor et al., 2018).

Different trials were conducted and showed a neuroprotective effect of albumin when given after subarachnoid hemorrhage as a promising tool to reduce morbidity and mortality (Suarez et al., 2012; Wang et al., 2017).

9. Myeloperoxidase (MPO)

MPO is an essential enzyme in the neutrophilic respiratory burst response to pathogens found in leukocytes' lysosomes (Lim et al., 2012). As a marker of inflammation increased, MPO was associated with poor prognosis in patients with aSAH. Lim et al. (2012) and colleagues noted that patients with symptomatic vasospasm compared to those without vasospasm had MPO levels of 115.5 ng/mL vs. 59.4 ng/mL respectively, 2 or more days before the vasospastic event.

Again, inflammation was highly suggested as the core cause of complications following subarachnoid hemorrhage.

10. Neuron-specific enolase (NSE)

NSE has been used as a biomarker for neuronal damage, and it is a glycolytic enzyme mainly localized in the neuron (Butterworth et al., 1996). Mabe and colleagues found that NSE levels in serum were significantly higher in patients with aSAH (29 patients) than patients without a normal range of 3.1 ± 0.6 ng/mL in serum. Also, the serum level increased with higher Fischer grades. Grades 2, 3, and 4 were 3.6 ± 1.3 , 5.6 ± 2.1 , and 7.6 ± 1.2 ng/mL, respectively, when taken within 3 days of aSAH (Mabe et al., 1991).

Kacira et al. (2007) and colleagues demonstrated an increasing level of serum NSE towards day 7 in 20 patients having aSAH, with values reaching 18.54 ± 19.86 µg/L compared with 5.94 ± 2.20 µg/L in a control group, and this was attributed to increased neuronal damage. Furthermore, Tawk et al. (2016) and colleagues involved 309 patients. They found that NSE level > 15 ng/mL is

considered relatively high and significantly associated with higher WFNS score, GCS, and H&H grade, concluding that NSE can be a potential predictor for poor outcome in aSAH. Prognostic studies can be based on the cutoff values found to incriminate this level officially in the clinical routine.

11. S100B

S100B is a calcium-binding peptide. Its production is carried out in astrocytes and has powerful paracrine and autocrine effects on neurons and glia (Rothermundt et al., 2003). It regulates the cytoskeletal structure and cell proliferation. At nanomolar concentrations, S100B improves neuronal survival and outgrowth in the cerebral cortex and decreases mitochondrial function loss during glucose shortage (Weiss et al., 2006).

In 55 patients with SAH, the mean daily value of S100B above 0.4 µg/L was higher in patients with high WFNS and Fisher scores (Weiss et al., 2006). S100B prognostic review of 74 patients with SAH also showed a significant correlation between high serum S100B (mean 0.17 µg/L) and poor prognosis as measured by GOS (Glasgow outcome scale), as well as cerebral infarction following aSAH (Moritz et al., 2010). Neither NSE nor S100B was correlated with the development of cerebral vasospasm (Moritz et al., 2010).

12. Conclusions

Aneurysmal Subarachnoid hemorrhage prognosis depends on the debilitating secondary outcome. Ongoing research to improve clinical management have surfaced to light in the past few years, mostly when numerous proteomics are studied to improve their predictive value regarding CVS and DCI after aSAH. Understanding the disease pathogenesis can open the opportunity to decipher how this proteomics relates to aSAH and thus translating them into clinical routine. Monitoring these biomarkers' trends allows the classification of patients in terms of poor outcome even before their neurological deterioration, and the prediction of various complications, hence identifying patients who need close monitoring and aggressive management.

Proteomics helped unravel many pathophysiologic processes, allowing medical interferences along several pathways to be used as future targeted therapy. Proteomics in the serum may help us better fathom the systemic response with its repercussions on the brain's local processes. A prospective clinical trial should be conducted to evaluate these proteomics' effectiveness, sensitivity and specificity, biokinetics, specific peaks, and half-lives. So far, not a single proteomic biomarker has proven to have sufficient sensitivity or specificity for DCI's reliable prediction. More proteomics should be complementary to concomitant neuroimaging and neuromonitoring techniques rather than replacing current modalities. DCI's complex pathophysiology makes it complicated to set a definitive pattern of biomarkers that can be a potential target for preventing complications.

Abbreviations

aSAH, Aneurysmal subarachnoid hemorrhage; CRP: C Reactive Protein; CVS: Cerebral Vasospasm; CSF: Cerebrospinal Fluid; DCI: Delayed Cerebral Ischemia; DIND: Delayed Ischemic Neurologic Deficit; ET-1: Endothelin-1; GOS: Glasgow Outcome

scale; NSE: Neuron-specific enolase; MPO: Myeloperoxidase; TRX: Thioredoxin; WFNS: World Federation of Neurological surgeons.

Author contributions

Shadi and Hani wrote the paper; Safwan, Charbel and Mohamad gathered the articles and did the tables. Dr Tarek Sunna reviewed the paper and edited it.

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Conflict of Interest

The authors declare no competing interests.

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